Inside JEB is a twice monthly feature, which highlights the key developments in the *Journal of Experimental Biology*. Written by science journalists, the short reports give the inside view of the science in JEB.

Inside JEB

CARBOHYDRATE: TOP HUMMINGBIRD FUEL

Picture by Kenneth Welch

As hummingbirds flit from flower to flower slurping up sugary nectar, more than 90% of the oxygen they breathe in is gobbled up by the mitochondria burning fuel to power their flight muscles. If a cell uses one 'unit' of oxygen to burn glucose, it will get 15% more ATP than if it used fat as fuel instead. However, researchers haven't yet made the connection between oxygen consumption and ATP production to fuel use in whole animals. As Ken Welch explains, 'hummingbirds are a unique model organism to investigate, since fasted birds make a rapid and predictable switch from fatty acid to carbohydrate metabolism after they begin feeding'. As they switch from burning fat to glucose, the birds' ratio of carbon dioxide production to oxygen consumption changes, so Welch and his mentor Raul Suarez predicted that as the birds switch between fuels, their rate of oxygen consumption would also go down (p. 2146).

The team fasted Anna's and rufous hummingbirds overnight to make sure that they were burning fat as the experiment started. Once released into the test enclosure, the birds eagerly fed from the tip of a syringe that dispensed sweet nectar within a respirometry mask. As they feasted on the nectar, air drawn through the mask passed into gas analysers which measured an increase in the birds' carbon dioxide production relative to their oxygen use. This told the team that the birds changed from burning fats to carbohydrate. 'The switch was very rapid, about 20 to 30 minutes', says Welch. During this time, the team calculated that the rate of oxygen consumption went down, showing that the birds needed less oxygen to make ATP after switching to sugar.

However, the team hadn't yet taken into account the fact that the sugary load caused the birds to gain weight, which in turn affected how much oxygen the muscles needed to produce enough power to keep the birds aloft. In between visits to the feeder, the hummingbirds sat on a perch which rested on a set of scales, allowing the team to measure their weight gain. Armed with their data, the team enlisted the help of hummingbird flight expert Doug Altshuler, who helped them to calculate how much oxygen the birds needed to produce a set amount of power during hovering flight. They used models which took into account the birds' changing mass, the forces generated by the wings during hovering and storage of energy by the birds' wings.

When they assumed that the wings stored energy between wing beats, they calculated that the rate of oxygen consumption went down by 16% as the birds switched from burning fat to glucose. If no energy is stored by the wings, the rate went down by 18%. These results were 'delightfully surprising', says Welch, since they were very close to theoretical estimates made by other researchers of different oxygen consumption rates when burning sugar or fat. This means that the hummingbirds need less oxygen to extract energy from carbohydrate, making it a more efficient fuel, and probably facilitating hovering at high altitudes where oxygen is scarce. Their quick fuel switch also shows that the hummingbirds are 'carbohydrate maximisers', says Welch; if carbohydrate is available, they will always use it over their precious fat reserves which will see them through leaner times.

10.1242/jeb.007963

Welch, K. C., Jr, Altshuler, D. L. and Suarez, R. K. (2007). Oxygen consumption rates in hovering hummingbirds reflect substrate-dependent differences in P/O ratios: carbohydrate as a 'premium fuel'. *J. Exp. Biol.* **210**, 2146-2153.

OXYGEN UPTAKE IN MITOCHONDRIA

If there's one thing that mitochondria thrive on, its oxygen. All of it is consumed by cytochrome oxidase, the last enzyme in the electron transport chain which drives ATP production. If cells relied on diffusion alone to supply them with their oxygen needs, then there would not be enough to keep up with demand. So oxygen carrying molecules, such as haemoglobin and myoglobin, evolved to transport oxygen to where it is needed. However as Jonathan and Beatrice Wittenberg explain, researchers know very little about the conditions necessary for oxygen to reach cytochrome oxidase (p. 2082).

As oxygen travels through the body it exerts a pressure in the mixture of gases in the lungs, or in solution, known as the partial pressure. Oxygen bound to haemoglobin in the blood diffuses down a steep pressure gradient into tissues as blood travels through capillaries. Next oxygen diffuses into the mitochondria. By reducing the oxygen pressure to levels below which mitochondria would not get enough oxygen without the help of haemoglobins, the Wittenbergs hoped to find the oxygen partial pressure necessary for oxygen uptake by mitochondria from hard working pigeon hearts. Also, would myoglobin in the heart muscle need to bind to mitochondria to deliver oxygen? To extract mitochondria for their study, the team delicately ground up the heart muscle tissue with a homogeniser and dissolved away the toughest tissue with enzymes; then, they released the mitochondria from the cell fragments and put them in a nourishing solution.

To show that myoglobin doesn't need to bind to the surface of mitochondria to deliver its oxygen, they used six different haemoglobins in the solution to deliver the oxygen: one each from horse, an insect, and soy bean, and three from molluscs. Each binds and releases oxygen at very different rates. Using a method called spectrophotometry, where a light is shone through biological samples and the light absorbed at each wavelength is measured, the team could tell how oxygenated the haemoglobins were since they absorb different light wavelengths depending on how much oxygen they are carrying. Despite differences in the speed with which oxygen bound to and was released from the haemoglobins, the mitochondria still took up oxygen at the same rate, showing that the haemoglobins didn't bind to the surface to deliver their cargo.

To find what oxygen partial pressure kept cytochrome oxidase functioning normally, they measured the saturation of each of the haemoglobins with oxygen and how it decreased as the mitochondria used oxygen up. From this they calculated oxygen pressure, which is directly related to haemoglobin saturation. When oxygen uptake was half its maximal rate, they found that the oxygen pressure at the surface of the mitochondria was very similar for all the haemoglobins, around 0.0053 kPa, despite their different reaction kinetics. This is much smaller than the pressure measured previously in working hearts, around 0.32 kPa. This means that even when a heart muscle is working flat out, such as during flight, the mitochondria will still have plenty of oxygen available to generate ATP.

Because oxygen uptake also levelled out as they increased the concentrations of the haemoglobins, the team suspect that there is just enough myoglobin present to support the cell, but not more, indicating that cells optimise oxygen delivery. 'The results were not unexpected', Jonathan Wittenberg explains. Despite this, he says, 'there is still a lot we don't understand about oxygen transport in heart and muscle'.

10.1242/jeb.007971

Wittenberg, J. B. and Wittenberg, B. A. (2007). Myoglobin-enhanced oxygen delivery to isolated cardiac mitochondria. *J. Exp. Biol.* **210**, 2082-2090.

KEEPING CRAYFISH UPRIGHT

Picture by Noriyuki Hama

If we didn't know which way was up, life would be very disorientating. While humans rely on a series of fluid filled canals and mineralised structures in the inner ear for balance, aquatic animals such as crayfish depend on organs at the base of each antennule called statocysts which respond to body tilting. Scientists already knew that an animal's behaviour affects how the nervous system responds to signals from the statocyst, but they had not measured this before in freely moving animals. Noriyuki Hama, Yoshikazu Tsuchida and Masakazu Takahata from Hokkaido University, Japan, took on the challenge, measuring the response of the nervous system to tilting in freely moving crayfish (p. 2199).

The team relied on an innovative and relatively new technique – optical telemetry – so that they could record from the crayfishes' nervous systems while they scurried around their tanks. They placed wire electrodes shaped like hooks around one of two identical large nerves which connect the crayfish's brain to the rest of the nervous system on each side of the body. Using this stable recording method, they could pick up activity in many of the individual neurons found in the nerve. Having fastened the electrodes securely into position they connected them to an infrared wireless transmitter which the crayfish carried on their backs. Four receivers placed at the corners of the tank picked up the signals and transferred them to a computer.

To investigate how the nervous system responds to tilting the team measured the activity of three neurons, which transmit feedback from the statocyst, as the crayfish sauntered across the floor of their tank, which was tilted to 10°. They found that not only did the activity of the neurons differ depending on which way the crayfishes' bodies tilted, but also on how they were behaving at the time. The first neuron, C_1, was more active when the crayfish walked up and down the slope than then they were resting. Recording from the nerve on the left side of the body, they found that when the crayfish walked across the slope such that the left side of the body was lower than the right, C_1 was more active. It was less active when the right side of the body was tilted lower. However, this response stopped when the abdomen was extended, but not when it was flexed under the body, showing that the part of the nervous system signalling the abdomen's position probably influences the activity of C_1.

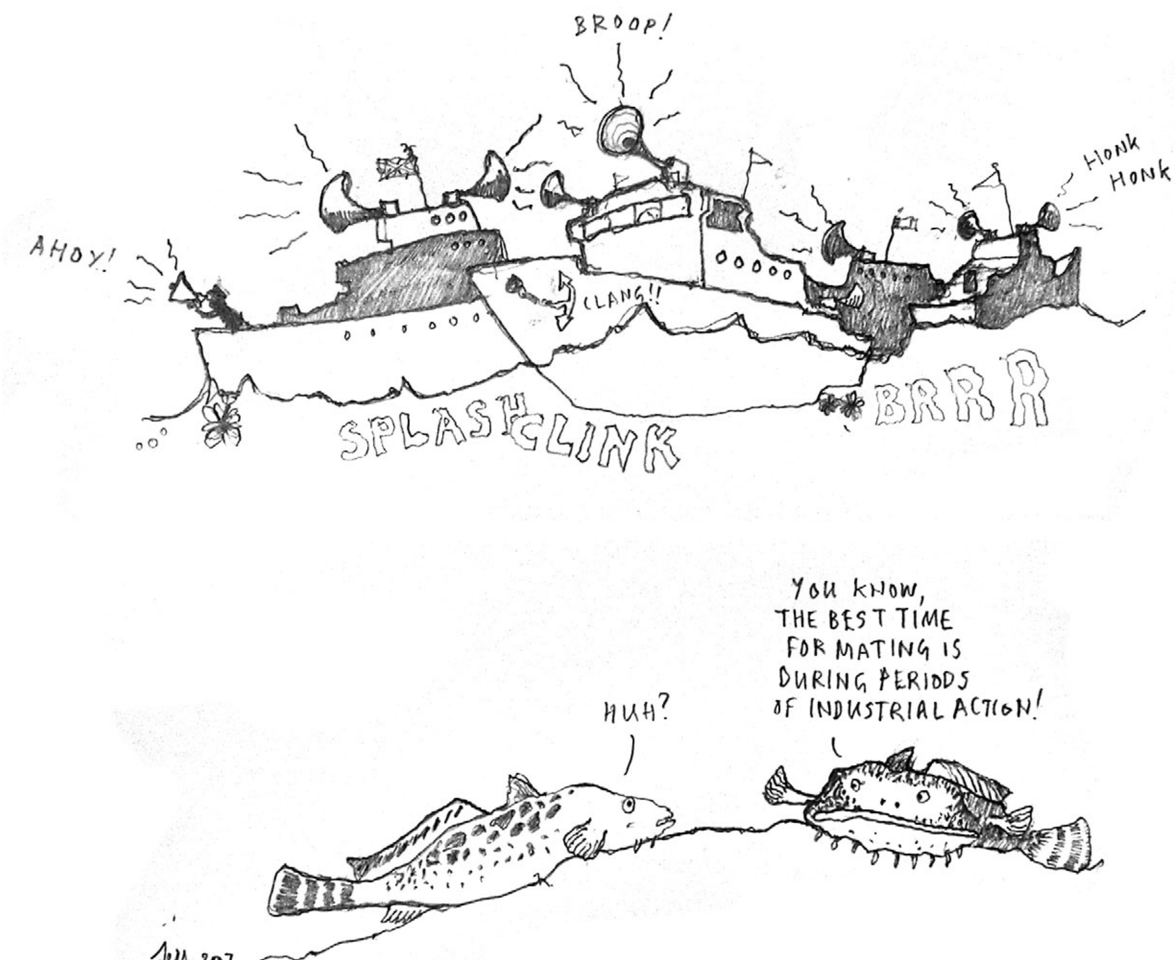
The second and third neurons, A and B, responded differently than C_1. Again recording from the left nerve, A fired when the body was tilted down to the left when the animal was at rest but stopped responding to the tilt when the crayfish ambled along. Flexing or extending the abdomen didn't have any effect either. In contrast, B responded to tilting to the right, both at rest and during walking. The activity was greater when the abdomen was held extended, but not when it was flexed, opposite to C_1's response.

So, perhaps not surprisingly, the nervous system responds to statocyst feedback in a varied and complex way in freely moving animals. Not only are neurons receiving input from the statocysts about body tilting, but also sensory input from the rest of the body and information about how the legs and abdomen are moving, which all work together to keep the crayfish upright.

10.1242/jeb.007989

Hama, N., Tsuchida, Y. and Takahata, M. (2007). Behavioral context-dependent modulation of descending statocyst pathways during free walking, as revealed by optical telemetry in crayfish. *J. Exp. Biol.* **210**, 2199-2211.

SHIPPING NOISE IS BAD FOR TOADFISH



Pete Jeffs is an illustrator living in Paris

To attract mates and deter other males from their precious nest sites, male toadfish, *Halobatrachus didactylus*, make a chorus of croaks, grunts and whistles. However their symphony can be disrupted by noise pollution from shipping, so Raquel Vasconcelos, Clara Amorim and Friedrich Ladich wanted to know how shipping noise affected toadfish living in the busy Tagus River estuary in Portugal (p. 2104). Measuring the sensitivity of the toadfishes' hearing in the lab, they found that when it was quiet the toadfish heard frequencies of around 50-200 Hz best, with hearing thresholds typically under 100 dB. Their hearing wasn't affected much when the team played them recordings of ambient noise from the estuary, but when they played estuary shipping noise to the toadfish, their hearing threshold increased by up to 36 dB. The boat noise was also most disruptive to their hearing between 50 and 200 Hz. Comparing recordings of the toadfishes' grunts and whistles with the hearing thresholds the team had measured under noisy conditions allowed them to check if the toadfish with higher hearing thresholds could still hear each other. They found that they couldn't hear each other as well with boat noise in the background, suggesting that toadfishes' acoustic communication is impaired when humans are around, which might impact on their survival and mean that they have to build their nests elsewhere.

10.1242/jeb.007997

Vasconcelos, R. O., Amorim, M. C. P. and Ladich, F. (2007). Effects of ship noise on the detectability of communication signals in the Lusitanian toadfish. *J. Exp. Biol.* **210**, 2104-2112.